REMARKS

Claims 1 to 9 are pending in this application. As requested in the Office Action, applicants have amended the specification to capitalize the terms SEPHADEX, GIGAPACK, and AMPLIFY. Applicants have amended claims 1 to 9 to clarify claim language and to change the claims from which claims 3, 4, and 8 depend. Certain specific fragments previously recited in multiple claims have been omitted from some claims merely to reduce redundancy. The amendments add no new matter to the present application.

Applicants acknowledge the Examiner's finding that claims 2 to 4, 7, and 8 are free of the prior art. Applicants respectfully submit that claims 1, 5, 6, and 9 are also free of the prior art, and that all of the pending claims are allowable for the reasons presented below.

I. The Invention

Several cellular proteins were found to bind hepatitis B virus (HBV) envelope prior to filing of the great-grandparent (U.S. Provisional Application serial no. 60/001,371) of the present application. However, none of those cellular proteins was shown to be an actual receptor for HBV (see the specification at page 2, lines 3 to 8). Applicants disclose two duck hepatitis B virus (DHBV) receptors (named "p170" and "p120" by applicants) in Pekin ducks, a model for the study of HBV infection in other animals (e.g., humans), and demonstrate that the two receptors specifically interact with discrete portions of the pre-S domain of the DHBV envelope protein. Applicants also demonstrate that synthetic pre-S polypeptides that block the binding sites on the p170 and p120 receptors interfere with DHBV infectivity (see the specification at page 42, lines 11 and 12). The present claims are directed to fragments of hepadnavirus pre-S proteins that, according to the specification, are capable of binding to receptor p120 and/or p170.

II. Rejections under § 112, second paragraph

Claims 1 and 6 were rejected as allegedly indefinite because, according to the Office Action (at page 3):

> The metes and bounds of the claimed polypeptide cannot be determined because it is unclear what specific sequences they are based upon. While the specification discloses two examples of amino acid sequences of pre-S proteins, it is unclear

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> whether either or both of these are the sequences of the claimed pre-S protein sequences or if other undisclosed sequences are also encompassed.

Applicants respectfully disagree and traverse this rejection. Claims 1 and 6 recite that the first amino acid sequence is identical to specific portions (e.g., amino acids 1 to 104) of a naturally occurring hepadnavirus pre-S protein. A skilled practitioner would know exactly what applicants mean by a "naturally occurring hepadnavirus pre-S protein." "Pre-S" in the context of HBV was well understood at the filing date, as evidenced, for example, by the frequent use of this term in the 1988 Cabezon et al publication cited by the Examiner. In addition, the term is precisely defined in the specification (at page 9, line 26 to page 10, line 3), which states:

> In all other contexts, a "naturally-occurring" hepadnavirus is intended to be synonymous with the sequence known to those skilled in the art as the "wild type" sequence, e.g., the wild type pre-S protein sequences shown in Figs. 16 and 17. If an amino acid sequence of a pre-S protein of a hepadnavirus that is derived from a natural isolate differs from the conventionally accepted "wild type" sequence, it is understood that the sequence of the natural isolate may be the proper comparison sequence for designing mutant polypeptides of the invention. The sequence of the natural isolate can be compared to the sequences cited herein to identify a receptor binding domain analogous to that of the DHBV pre-S domain.

Figs. 16 and 17, which are referred to in the above-quoted section of the specification, illustrate the amino acid sequence of wild-type DHBV pre-S protein and the amino acid sequences of wild-type pre-S proteins from twenty eight other strains of HBV, respectively.

Whether a sequence falls within the scope of claims 1 to 6 is determined by reference to naturally occurring (wild-type) hepadnavirus pre-S protein sequences. The specification provides at least twenty nine examples of naturally occurring pre-S protein sequences, and makes clear that other sequences not specifically disclosed in the specification may also fall within the scope of the claims. Based on the above, applicants submit that the metes and bounds of claims 1 and 6 are clear and that the claims are not indefinite. Thus, applicants request reconsideration and withdrawal of this rejection.

Claims 5 and 9 were also rejected as allegedly indefinite for recitation of the term "glutathione S-transferase." Specifically, the Office Action states (at page 3):

> [T]he metes and bounds of the claim cannot be ascertained absent specific recitation of the specific sequence of glutathione S-transferase encompassed

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> within the claims or in the alternative reference in applicant's disclosure to a sequence for glutathione transferase which is disclosed in the prior art.

Applicants respectfully traverse the present rejection for the following reasons. First, applicants submit that a specific glutathione S-transferase (GST) sequence need not be recited in the claims because any GST sequence can be included with the polypeptides recited in claims 1 and 6 (from which claims 5 and 9 depend, respectively). Skilled practitioners would recognize that GST sequences are routinely fused to proteins to facilitate protein purification and/or detection, and that no particular GST sequence is required for this purpose.

Second, applicants traverse because applicants do, in fact, make reference to a publication that describes an exemplary GST fusion protein system. This is the art-recognized GST system described by D.B. Smith and K.S. Johnson (Gene 67: 31 (1988)), which is incorporated by reference and referred to in the specification at page 56, lines 14 to 15, and page 7, lines 11 to 12.

Based on the above, applicants submit that the metes and bounds of claims 5 and 9 are clear and that the claims as filed are not indefinite. Thus, applicants respectfully request that the present rejection be reconsidered and withdrawn.

III. Rejections under § 112, first paragraph

Claims 1 to 9 were rejected under 35 U.S.C. § 112, first paragraph, for an alleged lack of written description. The Office Action indicates correctly that the claims are drawn to a genus, i.e., a genus of polypeptides that consist of an amino acid sequence identical to portions (e.g., amino acids 1 to 104 or 25 to 161) of a naturally occurring hepadnavirus pre-S protein and one or more heterologous sequences. However, the Office Action goes on to state (at page 4):

> There are two species of the claimed genus disclosed that are within the scope of the claimed genus, i.e., polypeptides comprising either the pre-S protein sequences of one particular strain of hepatitis B virus (HBV) or one particular strain of duck hepatitis B virus (SHBV) [sic] with glutathione S-transferase. The disclosure of one or two species may provide an adequate written description of a genus when the species disclosed are representative of the genus. However, the present claim encompasses numerous species that are not further described. There is substantial variability among the species.

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> One of skill in the art would not recognize from the disclosure that the applicant was in possession of the genus of polypeptides which comprises pre-S protein sequences from all strains of naturally occurring hepadnaviruses with a heterologous sequence or sequences.

Applicants respectfully traverse the present rejection, because the specification describes not two, but at least twenty-nine species of the claimed genus. Applicants again direct the Examiner's attention to Figures 16 and 17. Figure 16 illustrates the amino acid sequence of DHBV pre-S protein. Figure 17 illustrates the amino acid sequences of pre-S proteins from twenty eight strains of HBV that infect primates (see the specification at page 6, lines 26 to 28). A skilled practitioner would appreciate that sequences identical to fragments of any of these sequences could be fused to a heterologous sequence(s), e.g., GST sequences. It is further noted that, contrary to the Examiner's position, there appears to be little variability among the species of pre-S proteins illustrated in Figure 17. The Examiner is asked to provide evidence to support her contention that "there is substantial variability among the species."

Applicants submit that such an extensive disclosure of species constitutes a disclosure of a "representative number of species" of the claimed genus, as the quoted phrase is defined in the Manual of Patent Examining Procedure (*see* MPEP §2163(II)(A)(3)(a)(ii)). Specifically, applicants' disclosure is representative of the entire claimed genus of polypeptides, and the specification describes a variety of species sufficient to reflect the variation within the genus. Thus, applicants submit that claims 1 to 9 comply fully with the written description requirement and request that the present rejection be withdrawn.

Claims 1 to 9 were also rejected under 35 U.S.C. § 112, first paragraph, for an alleged lack of enablement. In support of this rejection, the Office Action states (at page 6)

> Given the teachings of unpredictability regarding the variability of the aa sequences of naturally occurring hepadnavirus pre-S proteins which are found in the art, detailed teachings are required to be present in the disclosure to enable the skilled artisan to make and use polypeptides corresponding to aa 1-104 or aa 25-161 of any naturally occurring hepadnavirus pre-S protein. Such teachings are absent. The specification discloses the sequences of the pre-S proteins for a single strain of HBV and for a single strain of DHBV. There is no disclosure of any pre-S sequences for any other strains or for any naturally occurring escape mutants.

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Applicants respectfully traverse this rejection because the specification does, in fact, provide the "detailed teachings" referred to in the above-quoted section of the Office Action. As discussed, the specification discloses pre-S protein sequences from at least twenty-nine strains of HBV. Also disclosed are specific fragments and individual amino acids of the DHBV and human HBV pre-S proteins that can interact with hepadnavirus receptors and interfere with HBV infectivity (see, e.g., the specification at page 6, line 1 to page 7, line 10). Although well within the ability of a skilled practitioner, the specification describes how to compare the DHBV pre-S protein sequence with other pre-S protein sequences to identify the important fragments and amino acids in those sequences (see, e.g., the specification at pages 6, lines 15 to 28, and Fig. 20). The specification also teaches that naturally occurring hepadnavirus pre-S sequences that differ from the disclosed wild type sequences (e.g., escape mutants) can be used as comparison sequences for designing the claimed polypeptides. The critical fragments and amino acids in the new sequences can be identified by comparison to the disclosed wild type sequences (see, e.g., the specification at page 9, line 29 to page 10, line 3).

Thus, contrary to what is asserted in the above-quoted section of the Office Action, applicants submit that the specification provides teachings sufficient to enable a skilled practitioner to make and use the present invention. The mere fact that there may exist a hepadnavirus pre-S protein sequence not specifically disclosed in the specification (e.g., that of some escape mutant) does not mean a skilled practitioner would be forced into undue experimentation to make and use the claimed polypeptides. Rather, in view of the teachings in the specification and the level of skill in the art, only routine experimentation would be required to make (and use) the claimed polypeptides based on the undisclosed sequence. Absent a need for undue experimentation, the present enablement rejection cannot stand. Thus, applicants respectfully request that this rejection be reconsidered and withdrawn.

IV. Rejections under 35 U.S.C. § 103

Claims 1 and 6 were rejected as allegedly obvious over Cabezon et al. (EP 0278940). Specifically, the Office Action states (at page 7):

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> Cabezon et al. teach polypeptides corresponding to the pre-S1 and pre-S2 regions of HBV. Cabezon teach that for the particular strain disclosed, the pre-S2 polypeptide is 163 aa in length, with the pre-S1 polypeptide corresponding to aa 1-108 and pre-S2 polypeptide corresponding to aa 109-163 (see page 1, lines 30-41). Cabezon et al. teach the HBV polypeptides fused with one or more heterologous sequences for preparation of vaccine compositions (see the abstract and second full paragraph under *Summary of the Invention*). Absent some evidence to the contrary, the polypeptides disclosed by Cabezon are obvious variants of the polypeptides of the claimed invention.

Applicants disagree with the above-quoted statement and respectfully traverse the present rejection. The polypeptides recited in claims 1 and 6 differ in length from the polypeptides described in Cabezon and are not made obvious in view of Cabezon.

Cabezon describes the use of full-length pre-S1 and pre-S2 protein sequences for preparation of vaccines against HBV. Cabezon does not teach, or even suggest, that a polypeptide consisting of an amino acid sequence identical to amino acids 1 to 104 or 25 to 161 of a hepadnavirus pre-S protein sequence fused to one or more heterologous sequences should be made. Nowhere does Cabezon suggest use of less than full length pre-S1 or pre-S2 for his vaccine compositions, and indeed the Examiner has not pointed to any such suggestion in Cabezon or elsewhere. The Examiner is reminded that a proper *prima facie* case of obviousness under U.S. law requires that the Examiner establish what in the prior art provides the necessary motivation to make the claimed invention. This has not been done here.

Applicants submit that the requisite motivation is not to be found in the prior art. Cabezon sought to make polypeptide vaccines. There is no reason to expect, *a priori*, that shortening the pre-S1 or pre-S2 polypeptide would produce a vaccine that works as well as the full-length polypeptides. Much less is there any motivation in Cabezon to make vaccines with the specific fragments (1-104 or smaller, or 25-161 or smaller) specified in the claims, whether or not combined with a heterologous sequence. Likewise, one of ordinary skill would find nothing in this reference to provide a reasonable expectation that a random fragment of pre-S1 or pre-S2 would make a successful vaccine. Again, the Examiner is reminded that without both a motivation and a reasonable expectation of success to be found in the art, the rejection cannot stand.

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Applicants' motive for making the claimed invention was one certainly not found in the art: to produce polypeptides that block the HBV binding sites on two cellular receptors, p170 and p120. Until Applicants carried out their experiments, it could not have been known what (if any) specific regions of the HBV envelope protein could be used for this purpose. Thus, no prior art could be said to supply what proved to be the motivation behind the present invention, much less an expectation that this invention would be successful.

Furthermore, Applicants take issue with the above-quoted statement that "Absent some evidence to the contrary, the polypeptides disclosed by Cabezon are obvious variants of the polypeptides of the claimed invention." This statement reflects a fundamental misapprehension of U.S. law. In determining whether the present invention is patentable under 35 U.S.C. §103(a) in view of a given reference, the Office is supposed to ascertain whether the claimed invention is an obvious variant of the prior art polypeptides, and not vice versa. Whether or not Cabezon's polypeptides are "obvious variants" of anything is irrelevant; the question is whether the claimed polypeptides are obvious. This is an important distinction. In formulating an obviousness rejection, one cannot start with Applicant's disclosure of specific fragments of the pre-S region, and look backward to see whether it would have been obvious to make Cabezon's prior art pre-S1 and pre-S2 polypeptides in view of Applicant's own disclosure. This appears to be what the Examiner has done.

For all of the above reasons, applicants request that the present rejection be reconsidered and withdrawn.

Claims 5 and 9 were rejected as allegedly obvious over Cabezon et al. in view of Khudyakov et al. (*Journal of Virology* 68/11:7067-74 (1994)). Applicants respectfully traverse this rejection for the reasons discussed below.

In support of the finding of obviousness, the Office Action states (at page 8):

> One of ordinary skill in the art at the time the invention was made would have found it *prima facie* obvious to have expressed the polypeptides disclosed by Cabezon et al. fused with glutathione S-transferase according to the teachings of Khudyakov et al.

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Applicants respectfully disagree for the following reasons. As discussed above, Cabezon does not teach or suggest the claimed polypeptide, nor provide any motivation for or expectation of success in making them. Khudyakov et al. does not supply what is missing from Cabezon, and indeed is cited not for any teachings about HBV, but rather for its teachings about GST fusions with other types of viral proteins.

Applicants submit that neither of the publications cited in the Office Action, singly or in combination, suggests developing the polypeptides of the present invention. Thus, applicants respectfully request that the present rejection be reconsidered and withdrawn.

CONCLUSION

Applicants submit that all claims are in condition for allowance, which action is requested. Attached is a marked-up version of the changes being made by the current amendment. The mark-up version is entitled "Version with Markings to Show Changes Made." Enclosed is a check for $460 for the Petition for Extension of Time fee for a three month extension. Please apply any other charges or any credits to Deposit Account No. 06-1050, referencing Attorney Docket Number 00786-287004.

Respectfully submitted,

Date: Nov. 21, 2002

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Version with Markings to Show Changes Made

In the specification:

The paragraph beginning at page 18, line 25 has been amended as follows:

--As a control for cell surface labeling, ^{125}I labeling of unfractionated liver proteins was performed. Frozen duck liver tissue was homogenized in lysis buffer and dialyzed overnight in 1.8% NaCl solution. Around 100 μg protein was labeled with 1 mci of ^{125}I using the iodogen method (Fraker et al., Biochem. Biophys. Res. Comm. 80:849-57, 1978) Labeled proteins were recovered by chromatography through a SEPHADEX™ [Sephadex] G50 column (Pharmacia). --

The paragraph on page 35, line 25 to page 36 line 14 has been amended as follows:

-- Construction of duck liver cDNA libraries. RNA was extracted from frozen Pekin duck liver with guanidinium thiocyanate (RNA isolation kit, Stratagene), and mRNA purified through an oligo dT column (POLY(A)QUIK™ [Poly(A)Quik] mRNA purification kit, Stratagene). Oligo dT primed and random primed lamda expression libraries were constructed using ZAP-cDNA synthesis kit and ZAP Express cDNA synthesis kit, respectively (Stratagene). For directional cloning, 1st strand cDNA synthesis was primed with oligo-dT (the primer also contains an XhoI site) using Moloney murine leukemia virus (M-MuLV) reverse transcriptase. After 2nd strand synthesis and addition of an EcoRI adaptor, the cDNAs were digested with XhoI and size-fractionated with SEPHACRYL™ [Sephacryl] S-400 column. Fractions 4 and 5 were combined and further electrophoresed in 1% agarose gel to isolate cDNAs greater than 1 kb in size. The purified cDNAs were ligated to EcoRI/XhoI double digested Lambda ZAP II vector. The ligation product was packaged into GIGAPACK™ [Gigapack]II Gold packaging extract (Stratagene), then plated in XL1-Blue MRF' host cells. The primary library was estimated to contain $6x10^6$ independent recombinants and has an average insert size of 1.9 kb. This oligo dT primed library was amplified once and stored in aliquots at 4°C (in chloroform) or -70°C (in DMSO). For construction of random primed library, 1st strand cDNA synthesis was primed by random hexameric oligonucleotides. The cDNAs were ligated with EcoRI adaptor, size fractionated with SEPHACRYL™ [Sephacryl] S-400 column, and cloned into EcoRI

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digested ZAP express vector (Stratagene). This random primed library has $6x10^6$ independent recombinants and an average insert size of 1.6 kb.--

The paragraph on page 19, lines 1 to 13 has been amended as follows:

-- Detection of pre-S binding proteins in labeled lysates. To reduce the levels of cellular proteins which bind to the GST component of the pre-S fusion protein or directly to the sepharose beads, labeled lysates were first preincubated twice at 4°C with a mixture of empty sepharose beads and GST-bound beads. The beads were washed extensively and used as a negative control in 8% SDS-PAGE. The precleared lysates were then incubated at 4°C for 6 to 16 hr with the specific GST-pre-S fusion protein. After extensive washing of the beads four times with lysis buffer, bound proteins were eluted from beads by heating to 95°C for 5 min, and separated on 8% SDS-PAGE under denaturing conditions. Proteins retained in the second preclearing reaction were run in parallel. The gel was fixed with 10% acetic acid, treated with AMPLIFY™ [Amplify] solution (Amersham), dried, and exposed. For experiments performed with the ^{125}I labeled proteins, the treatment step with AMPLIFY™ [Amplify] solution was omitted. Comparison of protein bands binding only to the pre-S fusion indicated the specificity of the interaction. --

In the Claims:

Claims 1 to 9 have been amended as follows:

1. (Amended) A polypeptide consisting of

a first amino acid sequence that is identical to (1) amino acids 1-104 of a naturally occurring hepadnavirus pre-S protein or (2) a fragment of amino acids 1-104 of the pre-S protein, provided that the fragment includes at least amino acids 80-102 of the pre-S protein; and

one or more amino acid sequences that are not identical to any part of the pre-S protein.

2. (Amended) The polypeptide of claim 1, wherein the first amino acid sequence is selected from the group consisting of amino acids [1-102, 25-102, 59-102, 80-102, 80-104,] 1-104, 25-104, 42-102, and 59-104 of SEQ ID NO:34.

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3. (Amended) The polypeptide of claim 1 [2], wherein the first amino acid sequence is selected from the group consisting of amino acids 1-102, 25-102, and 59-102[, and 80-102] of SEQ ID NO:34.

4. (Amended) The polypeptide of claim 1 [2], wherein the first amino acid sequence is amino acids 80-102 or 80-104 of SEQ ID NO:34.

5. (Amended) The polypeptide of claim 1, wherein the polypeptide comprises [one or more amino acid sequences include] the amino acid sequence of a glutathione S-transferase.

6. (Amended) A polypeptide consisting of

a first amino acid sequence that is identical to (1) amino acids 25-161 of a naturally occurring hepadnavirus pre-S protein or (2) a fragment of amino acids 25-161 of the pre-S protein, provided that the fragment includes at least amino acids 98-161 of the pre-S protein; and

one or more amino acid sequences that are not identical to any part of the pre-S protein.

7. (Amended) The polypeptide of claim 6, wherein the first amino acid sequence is selected from the group consisting of amino acids [92-161, 98-161,] 87-161, 26-161, 59-161, 71-161, and 80-161 of SEQ ID NO:34.

8. (Amended) The polypeptide of claim 6 [7], wherein the first amino acid sequence is amino acids 92-161 or 98-161 of SEQ ID NO:34.

9. (Amended) The polypeptide of claim 6, wherein polypeptide comprises [one or more amino acid sequences include] the amino acid sequence of a glutathione S-transferase.

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